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THE EFFECT OF FLUORIDE MOUTHRINSES ON BACTERIAL PLAQUE ACCUMULA--ETC(U)

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THE EFFECT OF FLUORIDE MOUTHRINSES ON BACTERIAL PLAQUE ACCUMULATION

Annual Report

September 1978

(for the period 1 October 1977 to 29 September 1978)

by

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Interference with bacterial adsorption due to fluorides was investigated *in vitro* by placing enamel cylinders in bacterial broth of *S. mutan*, while exposing the cylinders to four different fluorides twice a day. SnF_2 (250 ppmF^-) was noted to have the greatest effect on reducing bacterial colonization. A double blind clinical trial entailing 31 young adults was performed using the most effective regimen found from the previous experiments. Each trial period was of 5 days. The periodontal indices of the subjects on the SnF_2 rinse were found not to be statistically different from the placebo group, while the plaque score

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plaque weight, number of microorganisms per mg. and the total number of microorganisms was statistically reduced by the SnF_2 rinse.

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TABLE OF CONTENTS

Abstract

Recent investigations have shown that bacterial attachment to tooth enamel can be identified with electron microscopy and certain fluoride mouthrinses interfere with this adsorption of bacteria to enamel. The objective of this project was to identify the topical fluorides which alter bacterial adsorption to enamel and to clinically test these agents to develop a safe and effective regimen to reduce dental plaque accumulation.

Interference with bacterial adsorption to enamel due to fluorides was investigated in vitro by placing enamel cylinders in bacteriological broth with S. mutans, while exposing the cylinders to four different fluorides twice a day. The number of bacteria attached to enamel was used as a model to determine the type of fluoride that best altered bacterial sorption to enamel. Microbiologic counts were used to quantify the number of bacteria attached to the enamel cylinders. Bacterial attachment to teeth is in progress qualitatively judged by electron microscopic criteria.

A double blind clinical trial of cross-over-design entailing 31 young adult subjects was performed using the most effective regimen found from the previous experiments. After a thorough prophylaxis, all oral hygiene procedures were suspended for a one week period. Half of the subjects rinsed twice a day with stannous fluoride while the other half used a placebo rinse. After one week, periodontal indices, plaque indices, plaque weight, total microorganisms and number of microorganisms per mg were the criteria used to determine the efficacy of the experimental rinse. After indices were taken, both groups had a prophylaxis and continued for another week identical to the previous trial except for reversing experimental and control groups.

Introduction

An *in vivo* plaque model system of bacterial attachment to enamel has been developed by this author (Tinanoff, et al., 1976b). In this model, enamel cylinders are embedded into a Hawley appliance and worn for several days following which the cylinders are removed and the bacterial accumulation is determined. This *in vivo* plaque model system was used to investigate the effect of NaF_2 mouthrinses (both at 100 ppm F^-) on bacterial colonization of tooth enamel (Tinanoff, et al., 1976c). No apparent change in plaque formation was evident after two days when NaF was used once a day. The bacteria exhibited adherence to the enamel as well as to each other. Extracellular material, possibly involved in bacterial attachment, was present at the plaque-enamel interface. Yet, when this mouthrinse was used twice a day for a two-day period, there was some interference in the attachment of bacteria. The bacterial mass did not appear to be attached to the enamel, and even though the bacteria were found in clumps, they did not ultrastructurally display adhesion to each other.

Stannous fluoride mouthrinse had an even greater effect on the bacteria. When this mouthrinse was used once daily for a two-day period, the bacterial colonization of enamel was greatly reduced. If the mouthrinse was used twice daily, the enamel cylinders at two days were almost devoid of bacteria. From these findings it was apparent that there was more suppression of bacterial colonization on enamel by SnF_2 than by NaF.

Effects of tin ions on caries activity and bacterial concentration in saliva have been previously suggested. Muhler and Day (1950) reported that $SnCl_2$ could decrease dental caries. Stookey and McDonald (1974) found that 15 ppm tin resulted in significant cariostasis in rats. Andres, et al., (1974), concluded that the reduction of salivary bacteria due to higher concentrations of SnF_2 was most likely the result of the stannous ion rather than the fluoride ion.

However, in our studies the increased effectiveness of SnF_2 over NaF could not be explained by the antimicrobial properties of tin alone since: 1) no dramatic bacterial reduction on enamel surfaces was noted when $SnCl_2$ was used; 2) there was no evidence of bacteriolytic activity of SnF_2 mouthrinse; 3) preliminary microbiologic studies of NaF and SnF_2 at 100 ppm F^- (unpublished data) show no bacterial inhibition.

It appeared that the variation in microbial colonization on enamel due to different fluoride preparations may be the result of alteration of adhesive properties between enamel and bacteria, and between bacteria and bacteria. In the locations where bacteria were present on enamel after seven days of SnF_2 mouthrinses, these cells showed definite separation from each other. Even though it is difficult to elucidate mechanisms of action with confidence using electron microscopy, this finding suggests that either: 1) the repulsive charges on the bacteria may have been more intense; 2) the outer surface characteristics of the bacteria may have been changed; or 3) the bacteria may no longer be capable of producing extracellular adhesive/cohesive binding material. The apparent lack of attachment between bacteria may explain why characteristically only a single bacterial layer was formed on the seven-day sample upon fluoride treatment.

The effect of SnF_2 mouthrinse on initial bacterial colonization of tooth enamel has been investigated microbiologically (Gross and Tinanoff, 1977). In this study enamel cylinders, either embedded on the palate or on the buccal flanges of a Hawley appliance, were worn in two different individuals. Following a two-day period, the cylinders were removed from the appliance and the bacteria were dispersed, serially diluted and plated on blood agar and Mitis Salivarius agar. The SnF_2 mouthrinse greatly reduced the number of bacteria attached to the enamel in these early plaque samples. The total number of colony forming units was reduced 98.% when incubated aerobically, 96.8%, anaerobically; the number of streptococci were reduced 97.9% when incubated aerobically and 97.0%, anaerobically.

Investigations, screening nine fluoride mouthrinses for antiplaque properties

(Tinanoff, 1977) have further revealed that the type of fluoride may have an effect on bacterial accumulations on teeth. Scanning electron microscopy of enamel specimens worn for two days, during which time various fluorides were used as a rinse twice a day, showed that some fluoride solutions altered plaque formation, while others did not. Sodium fluoride, acidulated phosphate fluoride, and sodium monofluorophosphate did not appear to affect plaque formation. In contrast, stannous fluoride, sodium hexafluorostannate, and the amine fluorides reduced the number of bacteria dramatically.

Phase I In Vitro Studies

To further explore the effect of fluoride mouthrinses under this Army contract, we developed and used an *in vitro* model in which pure colonies of plaque producing microorganisms were incubated along with sterile surface enamel in bacterial broth. Observation and quantitation of suspected bacterial attachment modifiers (ie., certain fluorides) were performed using this model system.

The specifics of this procedure were as follows: extracted molars, derived from patients undergoing necessary, routine extractions, were collected and stored in tap water. Cylinders of surface enamel, 16.6 mm^2 were sectioned out of the smooth surfaces of these molars using a diamond drill (Pfingst and Co., New York, New York) with water coolant. The cylinders were cleaned with a slurry of pumice and then thoroughly washed in an ultrasonic cleaner with water. Wax was used to cover the enamel cylinder except for the surface enamel. These cylinders were then suspended by nylon monofilaments and placed in test tubes of 70% ethyl alcohol for 1/2 hour for sterilization. The cylinders were placed in sterile water for 10 minutes to dilute the alcohol on the enamel.

Thirty-six suspended enamel cylinders were divided into six groups of six cylinders. The cylinders in each group were suspended for one minute in 10 ml of the following agents: 1) distilled water; 2) NaF (100 ppm F^-); 3) SnF_2 (100 ppm F^-); 4) SnF_2 (250 ppm F^-); 5) NaF_6SN (100 ppm F^-); 6) $SNCl_2$ (100 ppm F^-).

Following exposure to these agents, each cylinder was immersed for one minute in 100 ml of sterile water to dilute the agents. The cylinders were then placed in 10 ml of Jordan's broth containing 5% sucrose. The test tubes from each group were innoculated with 0.2 ml of a 24 hour culture of S. mutans 10449.

The cylinders were incubated in the broth at 37^oC. At 12 hour intervals the cylinders were removed from the broth, suspended for one minute in H_2O or the appropriate fluoride, washed for one minute and replaced in their original tube containing Jordan's broth and the inoculum. To keep the cultures growing rapidly, all specimens were transferred to fresh Jordan's broth after 24 hours.

After a two-day incubation at 37^oC, two of the six cylinders from each group were used for electron microscopy which will be described in the following section. From the remaining four, one cylinder at a time was removed from the test tube. The wax was peeled off with its attached bacteria, thus leaving the enamel cylinder with plaque only on the surface-enamel side. Each specimen was then placed in a vial containing 2 ml of 0.05% yeast extract and sonified (Heat Systems, Model 185) such as to give maximal counts on agar plates. The dispersed plaque was serially diluted, and appropriate dilutions were placed on Mitis-Salivarius agar.

The cylinders with attached bacteria designated for electron microscopy from the previous experiment will be fixed in 2.5% gluteraldehyde in phosphate buffer and postfixed in 1% osmium tetroxide (Warshawsky and Moore, 1967). These cylinders were suspended in acid gel (0.1N HCl with 15% gelatin, pH 1) for four and one half hours to partially demineralize the enamel surface (Tinanoff, et al., 1976a). The specimens were then dehydrated in ethanol and embedded in Epon 812.

After polymerization, each specimen was sectioned 500 micrometers thick with a Gillings Hamco thin sectioning machine. After reembedding the sections, the enamel was ground from the inner surface on a sandpaper wheel,followed by hand grinding with 600 grit sandpaper until 5-10 micrometers of outer surface

of enamel remained. The specimens were then reembedded so that the Epon completely surrounded the enamel.

Due to time factor, these specimens have not yet been sectioned or observed in the electron microscope. But when specimens are prepared, thin sections for electron microscopy will be cut on an ultramicrotome using a diamond knife. These sections will be stained with aqueous uranyl acetate followed by lead citrate and the enamel-plaque interface will be examined with an electron microscope.

At least five areas from each specimen will be observed to minimize subjective misinterpretation. Comparison between samples will be made using six criteria: 1) actual bacterial attachment to enamel; 2) closeness of bacteria to enamel; 3) presence of extracellular material; 4) apparent affinity of bacterially produced extracellular material to enamel; 5) affinity and attachment of bacteria to each other; and 6) structural changes of bacteria, including cell walls.

Phase II In Vivo Study

Thirty one dental students were selected on the basis of having a complete dentition and volunteering for the study. From our *in vivo* studies, we settled on using SNF_2 at 250 ppm F^- as the experimental agent. The clinical trial was a cross over design so that each subject served as his own control. Chronologically the study was:

Week 1 — (Actually one week before the first trial). All subjects were thoroughly scaled and polished. Gingival inflammation and healing would thus be resolved by initiation of study.

Week 2 — Initially, periodontal index on six teeth (Ramfjord teeth). Then the teeth were again polished to reduce the plaque score to '0' (confirmed by staining). Half of the subjects were placed on the experimental rinse and half on the placebo rinse. Oral hygiene was suspended at this point. Students were seen twice a day for a supervised rinse of 10 ml of SNF_2. On day '5' plaque score (Shick and Ash. J. Perio. 32:346, 1961) and Periodontal index (Silness and Loe. Acta Odont. Scand. 22:121, 1964) on the "Ramfjord teeth" were performed. Additionally, all six teeth were stained and photographed, using a precision oriented instrument. After these procedures all the plaque from the six designated teeth was removed, weighed, and 1 mg. aliquots were diluted

with 2 ml phosphate buffer and sonified (Heat Systems, Model 185) for 15 seconds to suspend the bacteria to give maximal counts on agar plates. The dispersed plaque was serially diluted and the appropriate dilutions were plated on blood agar. After incubation, counts were made on plates showing 30-300 colony forming units. Using the weight of the total plaque collected for each individual and the number of colonies forming units per the aliquot, the total plaque recovery was calculated for each individual. This procedure for estimating plaque is essential since certain fluoride mouthrinses have been found to enhance a bacteria free deposit (pellicle) adherence to enamel which will interfere with plaque index and plaque weight measurements.

Week 3 Resumption of standard oral hygiene with no rinsing. This period reduced any possible carry over effect.

Week 4 Same as Week 2, but the groups were crossed over.

Week 5 Final cleaning for patients who desired it.

7 December 1977

Results of *In Vitro* Studies

PILOT STUDY I

Determining Surface Area on the Enamel Cylinder

Introduction

Uniformity of drilled enamel cylinders must be determined for future statistically valid results.

Materials and Methods

Ten (10) enamel cylinders previously drilled and cut, were randomly sampled from a population of thirty-five (35). Using a Boli gauge to measure their diameters, the results are tabulated below in millimeters.

Results

1.	5.5 mm	6.	5.4 mm
2.	5.5 mm	7.	5.6 mm
3.	5.5 mm	8.	5.4 mm
4.	5.6 mm	9.	5.4 mm
5.	5.6 mm	10.	5.5 mm

$$\begin{aligned} \bar{x} &= 5.5 \text{ mm} \\ r^2 &= \text{surface area of circle} \\ &= 3.14 \\ r &= 2.75 \\ (3.14)(2.75)^2 &= 23.7 \text{ mm}^2 = \text{mean surface area} \end{aligned}$$

Conclusions

The results show the cylinders were drilled and shaped to a uniform specification needed for equal comparison of experimental results in future tests.

PILOT STUDY II

Determine Minimal Lethal Concentration (MLC) of Disinfectant for Enamel Cylinders

Introduction

A variety of ways to sterilize the enamel cylinders was explored.

Ethylene oxide (gas): May be too reactant and the process too expensive for small surface volumes.

Autoclave (steam): May risk changes in enamel structure, 1% protein of the tooth's cystalline structure would be denatured. Alternate route which may be considered if necessary.

Alcohol (liquid): 70% ethyl alcohol as a disinfectant is the prime choice because of its ease of application and effective mode of antimicrobial potency.

Materials and Methods

Six (6) enamel cylinders were used in this time exposure experiment.

#1 control was noted to harbor organisms on the enamel cylinders when these specimens were placed in sterile water for 24 hours and then transferring to a Trypocase Soy Broth (TSB).

#2 control was a tube of TSB incubated for 24 hours to confirm satisfactory quality control of medium preparation.

Different time exposures for each enamel cylinder to the alcohol was used to determine a sterile environment. They were then transferred to TSB and the observed growth was recorded after 3 days incubation.

Results

Control #1: After 24 hours, heavily contaminated growth was present.

Control #2: After 3 days, no growth

Time Exposure to 70% EtOH.	Observation of TSB after 3 days at 37^{0}.
10 min.	No growth
1 hour	No growth
8 hours	No growth
72 hours	No growth

Conclusion

70% ethyl alcohol was shown to be the ideal choice. Being a surface tension reducer, a lipid solvent, and a coagulating agent, it has properties that contribute to bacteriocidal potency. For our experimental purposes, the enamel cylinders will be exposed to the disinfectant for 1 hour and rinsed in sterile deionized water for ½ hour to remove any residual effect.

PILOT STUDY III

Determining Optimum Setting for Sonifier Cell Disrupter

Introduction

In this experiment, a statistical approach to quantitation, using the Three-Tube Most Probable Number method (MPN) was used to determine the optimum setting needed to disrupt cell attachment from the enamel surface in the shortest period of time, thereby increasing cell numbers and decreasing possible cell injury.

Materials and Methods

Model #W185 Sonifier Cell Disrupter with a Branson converter attached to a micro-tip probe was incorporated to produce constant pulsating ultra-sonic sound waves. The time was set at a constant 15 seconds for all the experimental runs. Output control was the independent variable tested at different energy settings of 2, 4, and 6. A 24 hour culture of a streptomycin resistant mutant of *Streptococcus mutans* NCTC 10449 grown in Thioglycollate broth (BBL) for 24 hours at 37^o in 5% CO_2 atmosphere was serially diluted out to 10^{-7} org/ml. Dilution bottles contained 0.01 M phosphate buffer pH = 7.0 with 0.05% Yeast Extract (BBL).

Results

Output Control	3-Tube MPN Table	
2	$330 = 2.40 \times 10^5$ $310 = 0.43 \times 10^6$	$x = 3.4 \times 10^5$
4	$332 = 11.00 \times 10^5$ $311 = 0.75 \times 10^6$	$x = 9.3 \times 10^5$
6	$331 = 4.60 \times 10^5$ $310 = 0.43 \times 10^6$	$x = 4.3 \times 10^5$

Conclusion

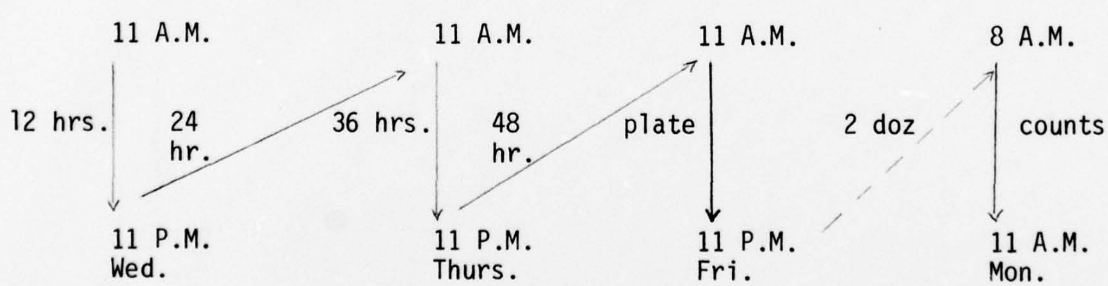
Output control 4 showing the highest mean organism count varifies this setting as the optimum condition for the release of viable organisms from the surface of the enamel cylinder.

Time Table Showing *In Vitro* Experiment during Intermittent Exposure to Fluorides.

Introduction

A well organized, smooth flowing long term experiment is conducive to accurate results and reproducibility, therefore, the schedule below will be strictly adhered to when repeating for experimental results.

Figure 1: Time Table



PILOT STUDY IV

Determine the Estimated Range of Serial Dilutions Needed for Accurate Plate Counts

Introduction

In order to insure accurate spread plate bacterial counts, colony forming units must be present in the 30-300 range according to the prescribed Standard Methods published by the A.P.H.A. Using the MPN method, the results should give an indication of the serial dilutions to be used for plating.

Materials and Methods

The MPN method was employed to estimate the dilution range. Five (5) enamel cylinders were grown in Jordans broth with 5% sucrose for 2 days at 37^{0} in 5% CO_2 atmosphere in the presence of *Streptococcus mutans* 10449, the conditions correlating exactly to that of the *in vitro* study proposed. An estimated educated guess to the base line was 10^{-5}, 10^{-6}, and 10^{-7}.

Results

Enamel Cylinder	3-Tube MPN Table
1	333 2.4×10^{7}
2	333 2.4×10^{7}
3	333 2.4×10^{7}
4	331 = 4.6×10^{6}
5	333 2.4×10^{7}

Conclusion

The *S. mutans* growth in the 1×10^{-7} dilutions seems to diminish in turbidity,

indicating a decrease in the amount of organisms present. Though it is not exactly clear as to where the ideal counts may occur, it does indicate 10^{-6}, 10^{-7}, and 10^{-8} dilutions should be satisfactory for the spread plate counts.

27 March 1978

PILOT STUDY V

Determine the Quantity of EDTA needed to Facilitate Sonication Effect

Introduction

The MPN method was used to compare two different molar concentrations of EDTA against that of water alone. This will test the effect of EDTA which acts as an anticoagulant by chelating divalent cations (forces which may be responsible for bacterial attachment in some degree), thereby increasing the number of colony forming units (CFU).

Materials and Methods

A 24 hour culture of Streptococcus mutans 10449 grown in Jordans broth with 5% sucrose at 37^{o} with 5% CO_2 atmosphere was serially diluted to 1×10^{-7} org/ml. The 3-tube MPN method used the dilutions of 10^{-5}, 10^{-6}, and 10^{-7} representing the base line.

Results

EDTA	3-Tube MPN Table
0.01 M	332 = 1.1×10^{7}
0.1 M	301 = 3.9×10^{5}
0.0 M (HOH)	331 = 4.6×10^{6}

Conclusion

The 0.1 M EDTA solution might have a toxic effect on bacterial growth as indicated by the MPN method. This finding is supported by information given to me in personal communication with Col. Arthur Gross, U.S.A.I.D.R. The water and 0.01 M EDTA are closely the same end result, with 0.01 M EDTA having a slight advantage. It has not been decided yet whether or not to add 0.01 M EDTA to the sample prior to sonication. Future trial runs are needed.

PILOT STUDY VI

Effect of Fluoride Compounds on Growth and Morphology upon Dental Plaque Microorganisms

Introduction

A visual qualitative analysis on the toxic effect of fluoride compounds upon microorganisms will be conducted using the concentration of 100 parts per million (ppm) fluoride. This will tend to give an overall view of the toxic suceptibility of individual organisms to fluoride compounds.

Materials and Methods

Spread plates of Mitis Salivarius Agar (MS) with 1% Potassium tellurite solution added, were divided into six equal flasks for each 100 ppm fluoride compound, which was Millipore filitered for sterility. Each pure culture was adapted to Jordans with 5% sucrose and swabbed on duplicate plates for each fluoride incorporated agar. A visual comparison rating of growth ranging from poor to excellent was recorded. Also recorded was any change in morphology which deviated from the control.

Results

Figure 1: F^- Compounds vs. Organisms
Absence of Growth at 37° for 2 days

	HOH	S_nF_2	NaF	$NaSnF_6$	TiF_4	$SnCl_2$
S. mutans 10449		-			-	
S. mutans 6715-13 Wt.						
S. sanguis 10558		-	-	-	-	
A. viscosus M-100	NG*	NG	NG	NG	NG	NG

*No Growth

Figure 2: Presence of Growth and Morphology Features with 5% CO_2 at 37^o for 2 days

S. mutans 10449

HOH:	Excellent
SnF_2:	NG
NaF:	Good
$NaSnF_6$:	Poor
TiF_4:	Fair
$SnCl_2$:	Very Good

S. mutans 6715-13 Wt.

HOH:	Excellent
SnF_2:	Fair
NaF:	Good
$NaSnF_6$:	Very Good
TiF_4:	Good
$SnCl_2$:	Very Good

S. sanguis 10558

HOH:	Excellent
SnF_2:	NG
NaF:	NG
$NaSnF_6$:	NG
TiF_4:	NG
$SnCl_2$:	Good

A. viscosus M-100

HOH:	Fair
SnF_2:	-
NaF:	Good
$NaSnF_6$:	Poor
TiF_4:	Very Poor
$SnCl_2$:	Poor

Conclusion

Actinomycetes viscosus is a strict anaerobic organism whose enzyme systems are inhibited by the presence of oxygen free gas because of its sensitivity to H_2O_2 produced by the Bio-oxidation process. Therefore, the aerobic and increased CO_2 tension was not the proper environment for growth. A Gas-Pak system (BBL) will be required for future studies.

However, Streptococcus mutans and Streptococcus sanguis are both microaerophilic organisms, able to function aerobically or anaerobically because their enzyme systems neither require nor utilize oxygen but need a lowered oxygen tension atmosphere to protect some of their enzyme which are sensitive to high levels of free oxygen. A 5% CO_2 atmosphere is ideal for this situation.

S. mutans 10449

Inhibited by SnF_2, no effect from $SnCl_2$. Good growth from NaF with liquid puddles surrounding colonies (glucan?).

S. mutans 6715-13 Wt.

Fair growth by SnF_2, no effect from $SnCl_2$. Good growth from NaF but no liquid puddles observed.

S. sanguis 10558

No growth on SnF_2, NaF, $NaSnF_6$, TiF_4. Good growth on $SnCl_2$.

SnF_2 has the greatest inhibitory effect in contrast to $SnCl_2$ having the least effect suggesting the tin ion may not play an important role in the inhibitory effect.

S. sanguis is shown to be sensitive to any type of fluoride compound, but this organism is not responsible for the formation of carious lesions.

Both S. mutans 10449, Bratthall serotype C; and S. mutans 6715-13 Wt., Bratthall serotype d, show an inhibitory effect to SnF_2. The mechanisms involved cannot be explored under this present grant.

PILOT STUDY VII

Effect of Fluoride Compounds upon Growth of Streptococcus mutans Serotypes in Liquid Medium

Introduction

A visual qualitative analysis on the toxic effect of fluoride compounds upon microorganisms was conducted using 100 ppm fluoride on all Bratthall serotypes a, b, c, d, and e. The purpose was to give an overall view of the susceptability of individual serotypes in a broth medium.

Materials and Methods

A pure culture representing each serotype was adapted to Jordan's broth containing 5% sucrose. The organisms below were used in the study.

Bratthall serotypes:

a	b	c	d	e
OMZ- 61	BHT	10449	6715-13	B-2

Each serotype was distributed (0.5 ml) into Jordan's broth containing one of the three 100 ppm fluoride compounds. A visual comparison of growth ranging from a scale of 1 (poor growth) to 5 (excellent growth) was performed (3 equaling the density of the control tube).

Results

Figure 1: F^- Compounds vs. Serotypes*

Bratthall serotype	SnF_2	$SnCl_2$	NaF	
a	3	3	2	
b	2	3	2	
c	3	4	3	
d	2	4	2	
e	3	3	2	
S. sanguis 10558	1	3	1	pancake growth

* Growth at 37^{o} for 24 hours.

Conclusion

This experiment demonstrated minor differences between serotypes when exposed to fluoride compounds in a liquid medium. The effect was not bacteriocidal at 100 ppm. There seems to be no stannous effect. This experiment closely agrees with the Streak Plate Method Experiment VI. S. sanguis again shows great sensitivity to any fluoride compound. Large amounts of precipitation were observed with $SnCl_2$ and SnF_2 in water, not in Jordan's broth.

An experiment using seeded plates of serotypes will be the final approach in differentiating them into separate groups by their growth reaction to fluoride compounds.

PILOT STUDY VIII

Effect of Fluoride Compounds upon Growth of Streptococcus mutans Serotypes Using the Spread Plate Method

Introduction

This was the final pilot study exploring the effects of fluoride compounds upon a colony growth and morphological characteristics. Using every fluoride compound available with every S. mutans serotype available, an overall view of the effects of fluoride on the same species with different serotypes was observed.

Materials and Methods

Spread plates of Mitis Salivarius Agar (MS) with 1% Potassium tellurite solution added were divided into six equal flasks for each 100 ppm fluoride compound, which was Millipore filtered for sterility. Each serotype was adapted to Jordan's with 5% sucrose and spread on duplicate plates for each fluoride incorporated agar. A visual comparison rating of growth from poor to excellent was used. A morphology which deviated from the control was another parameter. Actinomycetes viscosus M-100 was also used in the study. A Gas-Pak system (BBL) for an anaerobic environment was used.

Results

Presence of Growth and Morphology Features
with 5% CO_2 at 35° for 2 days observed at 20X

S. mutans 10449 serotype c

HOH:	Excellent
SnF_2:	Very poor
NaF :	Fair
$NaSnF_6$:	Fair
TiF_4:	Good
$SnCl_2$:	Very good

S. mutans 6715-13 serotype d

HOH:	Excellent
SnF_2:	Good
NaF	Very good
$NaSnF_6$:	Excellent
TiF_4:	Very good
$SnCl_2$:	Excellent

S. mutans OM2-61 serotype a

HOH:	Excellent
SnF_2:	Very poor
NaF:	Fair
$NaSnF_6$:	Fair
TiF_4:	Good
$SnCl_2$:	Very good

S. mutans BHT serotype b

HOH:	Excellent
SnF_2:	Poor
NaF:	Good
$NaSnF_6$:	Good
TiF_4:	Very good
$SnCl_2$:	Very good

S. mutans B-2 serotype e

HOH:	Excellent
SnF_2:	Fair
NaF:	Good
$NaSnF_6$:	Good
TiF_4 :	Good
$SnCl_2$:	Very good

S. sanguis 10558 A. viscosus M-100

HOH:	Excellent
SnF_2:	NG
NaF:	NG
$NaSnF_6$:	NG
TiF_4:	Fair
$SnCl_2$:	Good

A. viscosus M-100

HOH:	Excellent
SnF_2:	Fair
NaF:	Fair
$NaSnF_6$:	Fair
TiF_4:	Good
$SnCl_2$:	Fair

Conclusion

All of the S. mutans serotypes were affected by the F^- compounds to the same degree. Overall, SnF_2 seems to have the most effect on showing the rate of growth. $SnCl_2$ clearly does not interfere with growth rates. TiF_4 did not appear to affect growth rates to any great extent. Clearly, SnF_2 will have to be studied at different concentration levels. It may be feasible to eliminate TiF_4 substitute. This course of action would help improve the validity of the SnF_2 100 ppm observations.

PILOT STUDY IX

Practice Run Using Enamel Cylinders Exposed Only to Control

Introduction

Before committing a complete experimental run for this project, a decision was made to have a trial run exposing possible pitfalls in the design.

Materials and Methods

Schedule was followed according to the In Vitro Time Table previously explained in this report. Brain Heart Infusion Agar (BBL) was the medium used for spread plates. A pure culture of *Streptococcus mutans* 10449 was added to six (6) deionized water control tubes. Incubated aerobically at 37^{o} for 2 days.

Results

Counts on Duplicate Spread Plates using BHIA
Aerobically at 37^{o}, 2 days.

1	3,1	0,0	0,0
2	0,0	0,0	0,0
3	2,0	1,0	1,0
4	0,0	11,0	0,0
5	0,0	0,0	0,0
6	0,0	0,1	0,0

Colony Morphology

Enamel Cylinder

1 (3) gram + cocci, grapeclusters, white, 2 mm, smooth, circular, smooth, circular, raised entire.
(1) translucent, 1 mm, smooth, convex, circular, entire

3 (1) 1×10^{7} plate same as above
(2) puntiform, flat, dull, smooth, entire

4 (11) mixture of the same type of colonies experienced from #1 enamel cylinder.

Conclusion

S. mutans 10449 were not present on any of the plates. At first, we speculated our dilution factor was incorrectly calculated by the initial dilution of 1 ml removed from the total volume of the 2 ml sonified culture. This would have caused our plate counts to be over estimated, thereby having the plates spread to extinction. However, the few contaminants present on the spread plates were all strict aerobic bacteria when subcultured. It then occurred to us a lowering of the free oxygen tension necessary for microaerophilic growth was not present during its incubated time and although the organisms were present, the conditions were too toxic for growth.

Flow Chart In Vitro Main Study

Molars in H_2O → Drill $23.7\ mm^2$ cylinders → Slice enamel cylinders → Pumice 90^o turn →

Ultrasonic cleaner for 30 sec. in HOH → Hot wax covering → Attach nylon wire → 70 % Ethyl OH for 60 min.

→ Rinse (HOH/30 min)

6 tubes	6	6	6	6	6
Deionized HOH 1 min/10 ml	NaF (100 ppm) 1 min/10 ml)	SnF_2 (100 ppm) 1 min/10 ml)	$NaSnF_6$ (100 ppm) 1 min/10 ml)	TiF_4 (100 ppm) 1 min/10 ml)	$SnCl_2$ (100 ppm) 1 min/10 ml)

Deion. HOH 1 min/10 ml

↓

Jordans w/ 5% suc. 10 ml @ 37^o → Streptococcus mutans 10449 0.2ml of 24hr/37^o cult.

12 hrs. after

Suspend 1min/F^-

↓

Wash phos.buffer 1 min/100 ml

↓

(org.)Jordans 5% suc. $\xrightarrow{\text{24 hrs after}}$ Repeat with fresh Jordans — 36 hrs. after → Repeat

48 hrs. after

E.M. prep

2 cyl ← → 4 cyl

Wax removed → Sonified 2 ml yeast extract 0.05% → Serially dilute in yeast extr. $\xrightarrow{2dy\ 35^o}$ Plate count (30-300)

In Vitro Main Experiment

Introduction

The majority of laboratory time has been devoted to this main study whose purpose was to simulate rinsing with different fluoride agents twice a day. Because various fluorides and controls were used, this experimentwould be able to find the amount of reduction of various fluoride agents as well as to possibly reveal the mechanisms by which they inhibited colonization of bacteria on enamel. The first bacteria was *Streptococcus mutans* 10449,then *S. sanguis* 10558, and finally *Actinomyces viscosus* M100B was to be tested in the model system. The purpose and objectives of this experimentare further described in the introduction.

Materials and Methods

The flow chart on the following page describes in detail the steps involved in one experimental run. Due to a contamination problem which we were experiencing the following modifications of this procedure were performed: in experiment 2 and 3 streptomycin was added to the Mitis Salivarius plates so that no contaminant would be included in the counting of the organisms. (The Streptococcus mutans 10449 was a streptomycin resistant strain). The contaminant was noted in the broth along with the inoculum and was felt to be competing against the labeled streptococcus thus reducing its attachment and growth to the cylinders. Because of this, we adapted our *S. mutans* so that it would grow in broth containing streptomycin hoping to supress all other organisms. Since our contaminant was a Gram negative, aerobic, motile rod, we were not successful in reducing its prevalence by this method.

Colony Counts of S. mutans 10449 on Countable Spread Plates

Exp.	Dilution	(100 ppm) HOH	(100 ppm) NaF	(100ppm) $NaSnF_6$	(100 ppm) $SnCl_2$	(100 ppm) SnF_2	(250 ppm) SnF_2	(100ppm) TiF_4
		I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV	I II III IV
Exp. #1	10^{-7}	35 23 11 17	56 32 41	59 47 47 36	36 24 20 22	8 7 7 9	N/A	30 38 8 40
		25 18 11 27	44 23 34	– – – –	23 23 25 48	12 5 3 13		40 31 19 41
Exp. #2	10^{-5}	28 52 38 32	31 24 37 30	80 170 225 239	310 311 72 247	253 64 144 167	291 17 6 74	N/A
		22 59 36 22	29 31 44 27	50 192 241 69	370 194 78 285	320 51 119 102	310 15 5 62	
Exp. #3	10^{-5}	39 27 59 34	15 131 58 –	56 8 12 53	15 21 127 –	20 19 20 42	3 5 2 –	N/A
		16 23 79 45	25 124 38 –	107 7 8 59	11 23 101 –	9 18 11 31	2 4 2 –	
Exp. #4	10^{-4}	420 430 750 1300	89 35 24 16	40 26 43 17	35 12 5 55	4 111 5 26	9 45 40 5	N/A
		760 100 320 2230	42 22 33 14	51 36 36 5	29 14 7 63	0 131 3 40	22 81 26 8	

Statistical Analysis of S. mutans Intermittent Exposure - Experiment #1

```
VARIABLE  PLAQUE                                                      2/10/78    Acct: 6405

MULTIPLE RANGE TEST

DUNCAN PROCEDURE
RANGES FOR THE  .050 LEVEL -

          2.96   3.13   3.23   3.29   3.33

  HOMOGENEOUS SUBSETS   (SUBSETS OF GROUPS, NO PAIR OF W
                              SIGNIFICANT RANGE FOR A SUBSET

SUBSET  1

GROUP        GRP06        GRP01
MEAN          .6750       2.1000
- - - - - - - - - - - - - - - - -

SUBSET  2

GROUP        GRP01        GRP03        GRP04
MEAN         2.1000       2.7000       3.1750
- - - - - - - - - - - - - - - - - - - - - - - -

SUBSET  3

GROUP        GRP03        GRP04        GRP05
MEAN         2.7000       3.1750       3.6667
- - - - - - - - - - - - - - - - - - - - - - - -

SUBSET  4

GROUP        GRP05        GRP02
MEAN         3.6667       4.7250
- - - - - - - - - - - - - - - - -
```

6
1
3
4
5
2

```
ED

1:@RUN NDE,6405,HCDP  .  ED MUNSTERR
2:@SPSS.SPSS6
3:RUN NAME           TINNANOF
4:INPUT MEDIUM       CARD
5:VARIABLE LIST      FLUORIDE
6:INPUT FORMAT       FIXED(F1.0,F4.1 )
7:ONEWAY             FLOURIDE(1,6)/RANGES=DUNCAN(.05) /
8:STATISTICS         1
9:READ INPUT DATA
```

Group

```
               10:1 3.0 × 10^8
               11:1 2.1
1 Control      12:1 1.1
               13:1 2.2
               14:2 5.9
               15:2 4.7
2 NaSnF6       16:2 4.7
               17:2 3.6
               18:3 3.0
               19:3 2.3
3 SnCl2        20:3 2.9=3.5
               21:3 2.6=2.4
               22:4 3.5
               23:4 3.8
4 TiF4         24:4 1.4
               25:4 4.0
               26:5 5.0
5 NaF          27:5 2.8
               28:5 3.8
               29:6 0.9=1.1
6 SnF2         30:6 0.6
               31:6 1.0=0.5
               32:6 1.0
               33:FINISH

END ONSITE PRINTOUT ON FEBRUARY 10, 1978 AT 09:23:25
HCDP*NDETIN(1).
```

VARIABLE PLAQUE

ANALYSIS OF VARIANCE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F RATIO	F PROB.
BETWEEN GROUPS	5	35.5230	7.1046	10.386	.000
WITHIN GROUPS	17	11.6292	.6841		
TOTAL	22	47.1522			

GROUP	COUNT	MEAN	STANDARD DEVIATION	STANDARD ERROR	MINIMUM	MAXIMUM	95 PCT CONF INT FOR MEAN
GRP01	4	2.1000	.7789	.3894	1.1000	3.0000	.8606 TO 3.3394
GRP02	4	4.7250	.9394	.4697	3.6000	5.9000	3.2302 TO 6.2198
GRP03	4	2.7000	.3162	.1581	2.3000	3.0000	2.1968 TO 3.2032
GRP04	4	3.1750	1.2010	.6005	1.4000	4.0000	1.2639 TO 5.0861
GRP05	3	3.6667	1.1015	.6360	2.8000	5.0000	1.1303 TO 6.6030
GRP06	4	.8750	.1893	.0946	.6000	1.0000	.5738 TO 1.1762
TOTAL	23	2.8652	1.4640	.3053	.6000	5.9000	2.2321 TO 3.4983

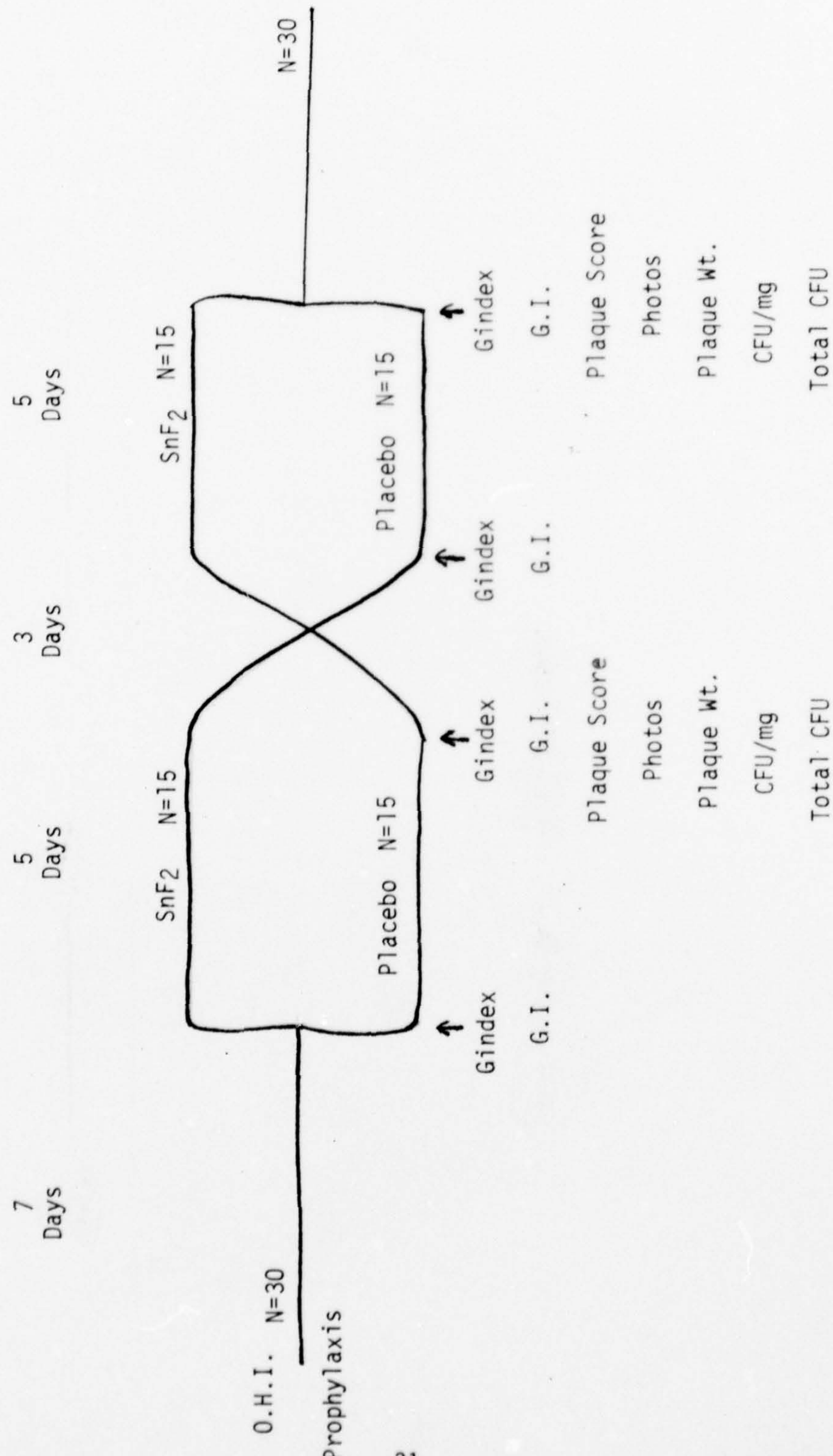
Conclusion

Our most successful experiment was #1 in which we had little contamination and visually there didn't appear to be any plaque formation on the SnF_2 exposed cylinders; whereas, all others had visual plaque formation. When the bacteria were sonicated, plated and counted, however, the reduction noted was not as great as expected and not statistically significant - see "Statistical analysis of S. Mutans Intermittent Exposure". We believed this may have been due to the possibility that sonication was more successful in disrupting cells into single colony forming units when exposed to SnF_2 than when exposed to other agents. Due to this thought, we did "Pilot Study V" to see if EDTA would facilitate breaking up "clumps of cells", thereby yielding higher counts. EDTA was found beneficial and utilized in the remaining experiments.

In experiments 2 and 3 we included another concentration of SnF_2 and eliminated TiF_4. There was variability on the counts from the cylinders in the same groups in the experiments, probably due to a contaminant which reduced the ability of S. mutans to proliferate in some cases. However, SnF_2 at 250 ppm appeared to reduce the number of bacteria on enamel by about ten fold. Experiment #4 with Streptomycin in the broth also had the same trend but contamination again led to high variability.

Since that time we think we have determined the source of the contamination. Enamel cylinders which are autoclaved, wax covered, and then placed in alcohol do not yield contamination; whereas enamel cylinders coated with wax and alcohol disinfectant do. We are currently in the midst of Experiment #5 with this new procedure.

Flow Chart -- In Vivo Study



Raw Microbiologic Data

(Total mg, CFU/mg, Total CFU) (* indicates tx group)

57 CO_2 @ 35°/2 da. Jac 6/26/78

		Sex	10^{-5}	10^{-6}	10^{-7}	mg.	CFU/ml	Total CFU/sam	Total CFU/mg	CFU/total wet plaque
1	~~D. Hochstein~~	F								
★ 2	D. Friel	F	TNTC	50-40	10-6	1.3	4.5×10^7	9.0×10^7	6.9×10^7	4.0×10^8
3	R. Weeks	F	TNTC	242-283	18-27	1.2	2.6×10^8	5.3×10^8	4.4×10^8	1.1×10^9
★ 4	D. Harinstein	M	TNTC	121-143	16-16	1.6	1.3×10^8	2.6×10^8	1.7×10^8	8.9×10^8
5	G. Robertson	F	TNTC	147-122	15-16	1.6	1.3×10^8	2.7×10^8	1.7×10^8	2.4×10^9
★ 6	A. Olsen	M	TNTC	154-147	16-20	1.5	1.5×10^8	3.0×10^8	2.0×10^8	4.3×10^9
7	P. Venezia	M	TNTC	62-60	5-1	1.2	6.1×10^7	1.2×10^8	1.0×10^8	1.3×10^9
★ 8	F. Inscgna	M	TNTC	101-126	13-19	1.7	1.1×10^8	2.3×10^8	1.3×10^8	5.3×10^8
9	M. Franks	M	TNTC	101-86	10-10	1.5	9.4×10^7	1.9×10^8	1.2×10^8	1.6×10^9
★ 10	S. Pear	M	TNTC	102-114	8-4	1.5	1.1×10^8	2.2×10^8	1.4×10^8	6.0×10^8
11	S. Holt	F	TNTC	110-114	5-11	1.5	1.1×10^8	2.2×10^8	1.5×10^8	2.4×10^9
★ 12	R. Annunziata	F	TNTC	83-85	13-12	1.6	8.4×10^7	1.7×10^8	1.1×10^8	6.4×10^8
13	K. Reilly	M	TNTC	173-175	17-17	1.8	1.7×10^8	3.5×10^8	1.9×10^8	8.3×10^8
★ 14	D. Wachnicki	M	TNTC	76-71	5-2	1.5	7.4×10^7	1.5×10^8	9.8×10^7	5.4×10^8
15	J. Gentile	M	TNTC	118-136	11-18	1.6	1.3×10^8	2.5×10^8	1.6×10^8	2.5×10^9
★ 16	R. Landesberg	F	TNTC	86-127	10-10	1.5	1.1×10^8	2.1×10^8	1.4×10^8	2.2×10^9
17	R. Meier	M	TNTC	260-347	34-25	1.6	3.0×10^8	6.1×10^8	3.8×10^8	8.0×10^9
★ 18	G. Ross	M	TNTC	143-176	4-11	1.3	1.6×10^8	3.2×10^8	2.5×10^8	1.3×10^9
19	R. D'Andrea	M	TNTC	72-115	10-12	1.6	9.4×10^7	1.9×10^8	1.2×10^8	1.8×10^9
★ 20	R. Viagrande	M	TNTC	96-98	13-11	1.7	9.7×10^7	1.9×10^8	1.1×10^8	2.0×10^9
21	T. Carta	M	TNTC	267-215	22-25	1.7	2.4×10^8	4.8×10^9	2.8×10^8	3.4×10^9
★ 22	D. Inouye	M	TNTC	126-145	10-4	1.7	1.4×10^8	2.7×10^8	1.6×10^8	1.5×10^9
23	D. Davis	F	TNTC	138-188	13-17	1.5	1.6×10^8	3.3×10^8	2.2×10^8	3.8×10^9
★ 24	G. Zabel	M	TNTC	162-161	18-15	1.5	1.6×10^8	3.2×10^8	2.2×10^8	2.2×10^9
25	G. Burbela	M	TNTC	171-174	10-15	1.3	1.7×10^8	3.5×10^8	2.7×10^8	1.9×10^9
★ 26	B. Ruby	M	TNTC	140-146	3-10	1.5	1.4×10^8	2.9×10^8	1.9×10^8	2.2×10^9*
27	A. Hawks	M	TNTC	91-109	4-3	1.4	1.0×10^8	2.0×10^8	1.4×10^8	3.2×10^9
★ 28	S. Ishizaka	F	TNTC	106-98	9-13	1.3	1.0×10^8	2.0×10^8	1.6×10^8	4.2×10^9
29	M. Rheaune	F	TNTC	183-183	19-19	1.7	1.8×10^8	3.7×10^8	2.2×10^8	3.2×10^9
★ 30	G. Naki	M	TNTC	76-65	7-4	1.5	7.1×10^7	1.4×10^8	9.4×10^7	1.2×10^9
31	J. Naki	F	TNTC	70-72	13-11	1.5	7.1×10^7	1.4×10^8	9.5×10^7	6.8×10^8

*retake of dry plaque

Raw Microbiologic Data

(Total mg, CFU/mg, Total CFU) (*indicates tx group)

[30] $y^1 + y^2 = \div 2 = CFU/_{ml} \times 2 = CFU/_{5cm} \div (mg) = CFU/_{mg} \times (tot.mg) = CFU/_{w.p.}$ 5% CO_2 @ 35°/2 da. DC 7/2/78

		10^{-5}	10^{-6}	10^{-7}	mg.	CFU/ml	Total CFU/total mg	Total CFU/mg	Total CFU/wt
★ 1 D. Hochst[illegible]	1	< 1.0 mg sample	– –	– –	–	–	–	–	–
2 D. Friel	2	TNTC	102-115	12-14	1.8	1.1×10^8	2.2×10^8	1.2×10^8	4.2×10^8
★ 3 R. Weeks	3	TNTC	41-49	5-6	0.6	4.5×10^7	9.0×10^7	1.5×10^8	2.3×10^8
4 D. Harinstein	4	TNTC	189-193	13-21	4.0	1.9×10^8	3.8×10^8	9.6×10^7	1.1×10^9
★ 5 G. Robertson	5	TNTC	112-126	11-14	1.5	1.2×10^8	2.4×10^8	1.6×10^8	1.6×10^9
6 A. Olsen	6	TNTC	83-94	7-9	1.7	8.9×10^7	1.8×10^8	1.0×10^8	1.5×10^9
★ 7 P. Venezia	7	TNTC	141-149	12-15	1.8	1.5×10^8	2.9×10^8	1.6×10^8	1.0×10^9
8 F. Insegna	ictures 8	TNTC	222-248	22-25	1.7	2.4×10^8	4.7×10^8	2.8×10^8	5.0×10^9
★ 9 M. Franks	9	TNTC	58-60	6-7	1.6	5.9×10^7	1.2×10^8	7.4×10^7	4.8×10^8
10 S. Pear	10	TNTC	115-121	8-9	(1.7) $\bar{x}$	1.2×10^8	2.4×10^8	1.4×10^8	3.3×10^8
★ 11 S. Holt	11	TNTC	118-148	9-13	2.3	1.3×10^8	2.7×10^8	1.2×10^8	9.1×10^8
12 R. Annunziat	12	TNTC	143-153	11-13	1.8	1.5×10^8	3.0×10^8	1.6×10^8	5.3×10^8
★ 13 K. Reilly	13	TNTC	43-61	1-3	1.7	5.2×10^7	1.0×10^8	6.1×10^7	1.8×10^8
14 D. Wachnick	14	TNTC	92-113	7-7	1.6	1.0×10^8	2.1×10^8	1.3×10^8	1.8×10^9
★ 15 J. Gentile	15	TNTC	103-113	4-7	1.3	1.1×10^8	2.2×10^8	1.7×10^8	3.0×10^9
16 R. Landesber	16	TNTC	137-178	13-15	1.6	1.6×10^8	3.2×10^8	2.0×10^8	6.3×10^9
★ 17 R. Meier	17	TNTC	104-113	12-16	1.8	1.1×10^8	2.2×10^8	1.2×10^8	1.9×10^9
18 G Ross	18	TNTC	91-95	10-11	1.6	9.3×10^7	1.9×10^8	1.2×10^8	1.5×10^9
★ 19 R. D'Andrea	19	TNTC	202-210	17-19	1.8	2.1×10^8	4.1×10^8	2.3×10^8	1.5×10^9
20 R. Viagrande	20	TNTC	218-266	19-22	1.5	2.4×10^8	4.8×10^8	3.2×10^8	3.1×10^9
★ 21 T. Carta	21	TNTC	71-89	8-10	1.5	8.0×10^7	1.6×10^8	1.1×10^8	7.0×10^8
22 D. Inouye	22	TNTC	237-240	19-22	1.7	2.4×10^8	4.8×10^8	2.8×10^8	1.8×10^9
★ 23 D. Davis	23	TNTC	102-115	10-18	1.6	1.1×10^8	2.2×10^8	1.4×10^8	7.3×10^8
24 G. Zabel	24	TNTC	205-207	18-19	1.5	2.1×10^8	4.1×10^8	2.7×10^8	1.9×10^9
★ 25 G. Burbela	25	TNTC	62-67	4-9	1.5	6.5×10^7	1.3×10^8	8.6×10^7	5.6×10^8
26 B. Ruby	26	TNTC	L.A.* L.A.	22-30	1.7	2.6×10^8	5.2×10^8	3.1×10^8	5.4×10^9
★ 27 A. Hawks	27	TNTC	165-179	9-11	1.5	2.5×10^8	5.1×10^8	3.4×10^8	2.7×10^9
28 S. Ishizaka	28	TNTC	156-170	13-17	1.8	3.3×10^8	6.5×10^8	3.6×10^8	6.7×10^9
★ 29 M. Rheaune	29	TNTC	119-125	8-13	1.4	1.2×10^8	2.4×10^8	1.7×10^8	1.8×10^9
30 G. Naki	30	TNTC	130-137	6-14	1.4	1.3×10^8	2.7×10^8	1.9×10^8	2.3×10^9
★ 31 J. Naki	31	TNTC	80-94	7-14	1.7	8.7×10^7	1.7×10^8	1.0×10^8	6.3×10^8

* = Laboratory [illegible]

Percent Reduction for Parameters on a Subject by Subject Basis

SUBJECT #	GINGIVAL INDEX	PLAQUE INDEX	PLAQUE WEIGHT IN MG	CFU/MG	TOTAL CFU
1	100.00 %	.00 %	-65.71 %	42.50 %	4.76 %
2	75.93	-11.54	42.31	65.91	79.09
3	88.73	6.67	53.45	-77.08	19.09
4	-158.62	-2.70	30.50	5.88	33.33
5	-23.53	-6.67	-48.95	-100.00	-186.67
6	-8.00	-13.79	47.15	-60.00	23.08
7	36.71	45.71	78.02	53.57	89.40
8	55.17	2.94	49.61	38.33	70.00
9	54.35	19.44	-75.00	.00	-81.82
10	-13.79	2.56	50.31	20.00	62.08
11	-253.85	-15.63	-90.62	31.25	-20.75
12	27.59	-9.68	30.23	67.89	78.31
13	.00	14.71	60.14	24.62	70.00
14	-8.00	12.50	-16.13	-6.25	-20.00
15	76.06	16.28	52.35	30.00	65.08
16	6.52	16.22	23.81	68.42	76.25
17	-8.70	-6.25	59.84	-108.33	13.33
18	-63.64	7.69	58.44	-91.67	16.67
19	-525.00	5.56	-82.11	65.63	35.48
20	100.00	7.89	44.54	60.71	79.41
21	100.00	17.95	-46.03	42.86	16.67
22	74.00	5.56	69.49	36.36	80.79
23	-97.62	10.53	-47.14	18.52	-15.79
24	91.30	2.63	8.45	-218.52	70.53
25	-1575.00	12.50	35.03	38.71	59.26
26	38.89	13.16	64.73	-142.86	15.63
27	-131.48	-11.43	-46.20	55.56	37.31
28	100.00	5.56	30.61	22.73	43.75
29	100.00	18.42	-10.17	50.53	47.83
30	100.00	9.09	13.89	-5.26	7.35
Mean	-54.73	5.86	12.49	1.00	28.98
SD	315.71	12.71	51.39	71.85	56.54
SE	57.64	2.3	9.38	13.11	10.32

Note -- Subject #1 was eliminated due to small amount of harvested plaque. Therefore subjects have been renumbered in this analysis, ie subject #2 is #1.

Summary Data for Percent Reductions

```
NORMAN  TINNANOFF                                                          29 AUG 78        PAGE   6

FILE   NONAME   (CREATION DATE = 29 AUG 78)

VARIABLE  PCT1        PCT REDUCTION GING INDEX MEAN

MEAN          -54.73256               STD ERROR    57.64227                 STD DEV     315.7197
VARIANCE      99678.93                KURTOSIS     17.04831                 SKEWNESS    -4.135071
RANGE         1675.000                MINIMUM      -1575.000                MAXIMUM     100.0000

VALID OBSERVATIONS -      30                    MISSING OBSERVATIONS -      0

- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

VARIABLE  PCT2        PCT REDUCTION PLAQUE INDEX MEAN

MEAN          5.862671                STD ERROR    2.320536                 STD DEV     12.71010
VARIANCE      161.5466                KURTOSIS     1.657827                 SKEWNESS    .6577000
RANGE         61.33929                MINIMUM      -15.62500                MAXIMUM     45.71429

VALID OBSERVATIONS -      30                    MISSING OBSERVATIONS -      0

- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

VARIABLE  PCT3        PCT REDUCTION PLAQUE WEIGHT IN MG

MEAN          12.49517                STD ERROR    9.383053                 STD DEV     51.39310
VARIANCE      2641.251                KURTOSIS     -.8584682                SKEWNESS    -.7174927
RANGE         163.6470                MINIMUM      -85.62500                MAXIMUM     78.02198

VALID OBSERVATIONS -      30                    MISSING OBSERVATIONS -      0

- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

VARIABLE  PCT4        PCT REDUCTION CFU MG 10**7

MEAN          1.000089                STD ERROR    13.11851                 STD DEV     71.85305
VARIANCE      5162.860                KURTOSIS     1.495342                 SKEWNESS    -1.488387
RANGE         286.9396                MINIMUM      -218.5185                MAXIMUM     68.42105

VALID OBSERVATIONS -      30                    MISSING OBSERVATIONS -      0

- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

VARIABLE  PCT5        PCT REDUCTION TOTAL CFU 10**7

MEAN          28.98186                STD ERROR    10.32454                 STD DEV     56.54983
VARIANCE      3197.884                KURTOSIS     5.612502                 SKEWNESS    -2.123374
RANGE         276.0667                MINIMUM      -186.6667                MAXIMUM     89.40000

VALID OBSERVATIONS -      30                    MISSING OBSERVATIONS -      0
```

Paired T Test for Variables in In Vivo Study

NORMAN TINNANOFF 29 AUG 78 PAGE 8

FILE NONAME (CREATION DATE = 29 AUG 78)

T - T E S T

VARIABLE	NUMBER OF CASES	MEAN	STANDARD DEVIATION	STANDARD ERROR	(DIFFERENCE) MEAN	STANDARD DEVIATION	STANDARD ERROR	CORR.	2-TAIL PROB.	T VALUE	DEGREES OF FREEDOM	2-TAIL PROB.
P1 PLACEBO TRT GING INDEX MEAN		.4667	.291	.353								
	30				.0810	.435	.079	.035	.855	1.02	29	.316
T1 SNF2 TRT GING INDEX MEAN		.3857	.333	.061								
P2 PLACEBO TRT PLAQUE INDEX MEAN		35.5000	3.803	.694								
	30				2.3333	4.436	.810	.344	.063	2.88	29	.007
T2 SNF2 TRT PLAQUE INDEX MEAN		33.1667	3.940	.719								
P3 PLACEBO TRT PLAQUE WEIGHT IN MG		12.5767	6.645	1.213								
	30				3.2700	6.609	1.207	.445	.014	2.71	29	.011
T3 SNF2 TRT PLAQUE WEIGHT IN MG		9.3067	5.831	1.065								
P4 PLACEBO TRT CFU MG 10**7		20.4367	9.426	1.721								
	30				3.0833	16.336	2.982	.090	.635	1.03	29	.310
T4 SNF2 TRT CFU MG 10**7		17.3533	14.221	2.596								
P5 PLACEBO TRT TOTAL CFU 10**7		259.3000	194.765	35.559								
	30				117.2333	174.103	31.787	.458	.011	3.69	29	.001
T5 SNF2 TRT TOTAL CFU 10**7		142.0667	107.643	19.653								

Preliminary Conclusions

In Vitro Studies

From our pilot studies and main *in vitro* experiments we have noted several generalizable findings concerning the interaction of fluorides with bacteria. The only fluoride that shows any reduction on growth and attachment of bacteria to enamel is SnF_2. This reduction appears to be in the range of one log. More experiments are in progress to become more positive about this effect. It appears that the Sn^{++} ion is partially responsible for this effect since some experiments show $SnCl_2$ to alter bacterial growth - but they are not as great as those effects found with SnF_2. SnF_2 at 250 ppm appears to be more effective than SnF_2 at 100 ppm. We used this finding to select the concentrations for our *in vivo* study. Preliminary results also show that SnF_2 will be more effective in reducing the population of *S. sanguis* than *S. mutans* or *A. viscosus*. Since the *in vivo* phase is essentially completed, we are currently devoting a complete effort to continue these *in vitro* studies so that the effects of fluoride on these different bacteria can be determined with certainty. Electron microscopy of the specimens derived from experiment #1 is also in progress.

In Vivo Study

The clinical study to test the efficacy of SnF_2 mouthrinse went extremely well. All 31 subjects who began the study completed the experiment. There was complete cooperation with the suspension of oral hygiene, attending the supervised rinsings twice a day, and coming to all examinations. Out of a total of 124 examinations and 496 supervised rinses only 3 rinse appointments were missed. We felt that one missed rinse appointment per subject would not alter the results, so these subjects were not eliminated. One subject was elmininated (subject 1), however, due to the fact that very little plaque could be harvested

from her mouth - possibly due to lack of cooperation with suspension of oral hygiene. Fortunately, there were no errors in the battery of clinical and microbiologic indices and all data were felt to be valid.

From the variables tested the following mean percent reductions were found with the SnF_2 rinse: Plaque Index - 5.8%; Plaque Weight (in mg.) - 12.5%; Colony Forming Units/Mg - 1%; Total Colony Forming Units/6 Teeth - 28.9%. One unexpected finding was that the gingival index mean was found to be lower when subjects were on the placebo agent. This may be due to one subject, however, who had an unexplained 1575% increase in the plaque index at the end of the SnF_2 rinse period. From the variable examined, the following reductions were statistically significant: Plaque Index, $P < .007$; Plaque wet weight, $P < .011$; Total Colony Forming Units, $P < .001$. (Because there has not as yet been hand confirmation of the computer print outs, these data are still felt to be tentative).

Data analysis is still in progress with the following tests to be run: (1) Correlation between parameters, (2) Inter examiner correlations for Gindex (a gingival index parameter), (3) Weekly differences between groups, (4) Non-parametric comparisons of the 372 photos taken of the plaque disclosed teeth. This data will be forwarded when compiled.

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Papers Published:

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2. Tinanoff, N. Use of scanning electron microscopy to screen fluorides for antiplaque properties. Scanning Electron Microscopy 2: 1003-1007, 1978.

Papers in Press:

1. Tinanoff, N., Tanzer, J.M., and Freedman, M.L. In vitro colonization of Streptococcus mutans on enamel. Infec. Immum. Sept, 1978.

2. Tinanoff, N. and Tanzer, J.M. Attachment of plaque forming bacteria to enamel. J. Ped. Dent. Jan-Feb., 1979.

Papers Submitted:

1. Tinanoff, N., Hock, J., Camosci, D. and Hellden, L. Clinical trial of SnF_2 mouthrinse as an antiplaque agent. Proc. Int. Assoc. Dent. Res. New Orleans, 1979.

2. Tinanoff, N. and Tanzer, J.M. Attachment of plaque forming bacteria to enamel. Proc. Int. Assoc. Dent. Res. New Orleans, 1979

3. Weeks, D.L. and Tinanoff, N. Current status on the effects of SnF_2 on dental plaque. J. Prev. Dent.

Appendix A - Consent Form

SCHOOL OF DENTAL MEDICINE

UNIVERSITY OF CONNECTICUT HEALTH CENTER

PATIENT CONSENT FORM

Investigator: Norman Tinanoff, D.D.S., M.S.

It has recently been suggested that some commercially available fluoride mouthrinses might inhibit bacterial accumulation of teeth as well as decreasing enamel solubility to make teeth less susceptible to dental caries. We would like your permission to take part in a clinical research experiment to measure the possible anti-plaque effects of one of these fluoride mouthrinses.

Procedure: Initially, a thorough cleaning (dental prophylaxis) will be performed on your teeth. They you will be asked to suspend personal oral hygiene procedures (toothbrushing, flossing, mouthrinsing) for four days. At this point, the plaque accumulation will be scored and you will receive another cleaning. For the next four days you will suspend oral hygiene procedures, but, this time, use a designated mouthrinse twice a day. After this four day period, the plaque will be scored, your teeth will be cleaned and, again, you will use a mouthrinse for four days, without any other oral hygiene procedures.

Risks: Besides the possibility of some slight staining of the teeth, which can be easily removed, no side effects from the mouthrinses have been reported.

Benefits: Each participant will receive three dental cleanings: One at the beginning of the study, one at the initiation of the second phase and one at the beginning of the third aspect of the study. Furthermore, it has been well established, the mouthrinses used are beneficial in reducing dental caries.

Provided there has been compliance with all phases of the study, you will receive $50. as a reimbursement for the time taken by the study.

I consent to participate in the study.

Date: ______________________ Signature of patient: Marc Franks

Witness: Peter Venegia

Marc Franks
201 Regan Rd. Apt 4A
Vernon, Conn.

871-2556

Appendix B - List of Participants

NAME	ADDRESS	S.S.#	SIGNATURE
Denise Hochstein	88 Brittany Farms Road, Apt. 103J (103J) New Britain, CT 06053 224-8855	043-40-8571	Denise Hochstein
Daria Friel	45 Kirkwood Road West Hartford, CT. 06117 233-3774	049-46-0546	Daria M Friel
Robin Weeks	778 Downey Drive 423-2283 Manchester, CT 06040 289-4629 c/o George Patros 41 Summit St. Willimantic CT 06040	044-50-0477	Robin A. Weeks
David Harinstein	357 Brittany Farms Road New Britain, CT 06053 223-3539 Apt. 129H	047-38-2375	David Harinstein
Gillian Robertson	Hartford Seminary, Box 110 55 Elizabeth Street Hartford, CT 06105 233-9389	045-42-4055	Gillian Robertson
Allan Olson	194 Munson Road Wolcott, CT 06716 879-4088	353-36-7889	AWOlson
Peter Venezia	87 Evergreen Avenue Hartford, CT 06105 233-8121	047-44-5297	Peter Venezia
Fred Insogna	Farmington House Apts., Apt. G 418 Farmington Avenue New Britain, CT 06053 229-7316	047-50-2316	Fred Insogna
Marc Franks	201 Regan Road, Apt. 4A Vernon, CT 06066 871-2556	044-44-4439	Marc Franks
Steven Pear	Falls Road 632-1212 or Moodus, CT 873-9267	040-48-9291	Steven Pear
Sheryn Holt	172 Allen Street, Apt. A-4 New Britain, CT 06053 223-7856	044-52-8879	Sheryn Holt
Regina Annunziata	27 Norwood Road West Hartford, CT 06117 523-8626	043-54-1746	Regina Annunziata
Kevin Reilly	462 Pine Street Waterbury, CT 06704 756-1097	047-50-1744	Kevin E Reilly
David Wachnicki	950 Farmington Avenue, Apt. C-7 New Britain, CT 06052 223-4886	041-50-6737	J Wachnicki
Joe Gentile	~~1200 Hillcrest Avenue~~ 55 Brittany Farms Rd ~~West Hartford, CT~~ ~~249-4857~~ New Britain, Ct 229-2569	044-54-6347	Joseph Gentile
Regina Landesberg	~~45 Stauton Circle~~ 61 TALCOTT FOREST RD Farmington, CT 06032	047-48-6589	Regina Landesberg
Rich Meier	152 Hawthorne Street Manchester, CT 06040 649-5108	048-46-8223	Richard W. Meier

NAME	ADDRESS	S.S.#	SIGNATURE
GARY Ross	19 Lake Shore Drive, Apt. B-1 Farmington, CT 06032 677-4895	101449566	Gary Ross
Ron D'Andrea	~~Talcott Forest Apts., Apt. 4F~~ ~~Farmington, CT 06032 677-7998~~ 155 Kathrine Dr. Hamden, Conn. 06514 248-9057	SS# 047509946	Ronald J. D'Andrea
Rich Viagrande	Country Club Estates, Apr. 310 2090 Stanley Street New Britain, CT 06053 224-9791 87 LAURA LA. NEW HAVEN CT	041-50-0931	
Tris Carta	6A Talcott Forest Road Farmington, CT 06032 677-4931	045-48-3604	Tris J. Carta
David Inouye	~~UCHC Box 216~~ 62 Montauk St Fairfield, CT 06430	049 52 8383	David Inouye
Donna Davis	82 Brittany Farms Road, Apt. 336J New Britain, CT 06053 223-6451	354-48-4027	Donna Davis
Gary Zabel	163 Camp Street Plainville, CT 06062 747-6576	049-52-2808	Gary H. Zabel
Greg Burbela	127 Perry Street, UCHC Box 199 Unionville, CT 06085 673-9471	041-48-5506	Gregory Paul Burbela
Bob Ruby	154 Colony Avenue Trumbull, CT 06611 268-5581	047-38-1117	Robert J Ruby
Andrew Hawks	150 Merigold Drive New Britain, CT 06053 223-8670	485-52-4790	Andrew Hawks
Sally Ishizaka	~~49-D Grandview Drive~~ ~~Farmington, CT 06032 677-7775 X2650~~ 1240 Robin Hill Rd. Media, Pa. 19063	175-46-9243	Sally J. Ishizaka
M. Rheaune	23 Kingston Street West Hartford, CT 06119 233-6309	018-48-4474	Michelle Rheaume
Jill Nuki Guy Nuki	90 Fox Den Road Avon, CT 06001 673-4425	484707088 484707088	J Nuki Guy Nuki

Appendix C - Schedule for In Vivo Study

THE UNIVERSITY OF CONNECTICUT
HEALTH CENTER

June 6, 1978

Dear

Enclosed is your schedule for the anti-plaque mouthrinse study.

You first visit is Monday, June 12th at __________. Please be in the waiting room of Dental Clinic #9 (Emergency and Screening) a few minutes before this time so we can keep on schedule. This visit will take approximately 45 minutes and will include getting your teeth cleaned and giving you oral hygiene instructions. It is important that you remember this visit since we will not be able to give you a reminder at a lecture!

Your next visit will be Monday, June 19th at __________ in the same clinic. At this time we will reduce your plaque to 0 and start you on our rinses.

Twice a day supervised rinsing will be performed in room 6037. You can show up any time between 7:30 - 8:00 AM and then again between 5:00 - 5:30 PM.

Friday, June 23rd, at __________ will be your first examination during which time plaque will be assessed by several parameters.

After this appointment you resume normal oral hygiene until Monday, June 26 at __________ where we will again reduce your plaque to 0 and start you on a different rinse.

Friday, June 30th at __________ is your last appointment for plaque indices.

Please remember we need your cooperation with all appointments and instructions. Reimbursements of $50 necessitates making all visits so mark them down on your calendar.

Thank you again for volunteering for this study. I think it will be educationally satisfying to participate in this study.

We will try to compile the data in time so that we can send you the results of the study at the same time we mail your check.

If you have any questions, you can contact me at 674-3158.

Regards,

Norman Tinanoff

Norman Tinanoff, D.D.S., M.S.

SCHOOL OF DENTAL MEDICINE
FARMINGTON, CONNECTICUT 06032

Appendix D - Clinical Chair Assignment _In Vivo_ Study

June 12

A	1:00	(2)Denise Hochstein, (3)Daria Friel, (4)Robin Weeks, (5)David Harinstein (6)Gillian Robertson, (7)Allan Olson
B	1:45	(2)Peter Venezia, (3)Fred Insogna, (4)Marc Franks, (5)Steve Pear, (6)Sheryn Holt, (7)Regina Annunziata
C	2:30	(2)Kevin Reilly, (3)David Wachnicki, (4)Joe Gentile, (5)Regina Landesberg, (6)Rich Meier, (7)Gary Ross
D	3:15	(2)Ron D'Andrea, (3)Rich Viagrande, (4)Tris Carta, (5)David Inouye, (6)Donna Davis, (7)Gary Zabel
E	4:00	(2)Greg Burbela, (3)Bob Ruby, (4)Andrew Hawks, (5)Sally Ishizaka, (6)Michelle Rheaune, (7)Guy Nuki, (8)Jiil Nuki

June 19

1:00	A
1:45	B
2:30	C
3:15	D
4:00	E

June 26

3:30	A
4:15	B
5:00	C
5:45	D
6:30	E

June 23

3:30	A
4:00	B
4:30	C
5:00	D
5:30	E

June 30

1:00	A
2:15 ~~1:30~~	B
3:30 ~~2:00~~	C
4:45 ~~2:30~~	D
6:00 ~~3:00~~	E

Appendix E - Distribution of Assignments - In Vivo Study

June 5 Letters out to students giving them appointment times
June 9 Followup phone call

June 9 Organizational meeting 3 PM Rm 6040
Tinanoff Hellden Rosania Shait Lamont Weeks Mckee Hock Dental Ass

June 12 Scaling, pumice prophylaxis, OHI
Appointments at 1:00, 1:45, 2:30, 3:15, 4:00 6 students each

June 19 & 26
1. Gindex Rosania and Shait
2. Gingival index Hock
3. Light pumicing
4. Check for 0 plaque
5. First supervised rinse

Lamont, McKee, Weeks, Hock, Hellden, Rosania (items 3–5)

June 19 appointments at 1:00, 1:45, 2:30, 3:15, 4:00
June 26 appointments at 3:30, 4:15, 5:00, 5:45, 6:30

June 23 & 30
1. Gindex - Rosania and Shait
2. Staining and Plaque score - Hellden and McKee
3. Photos - Tinanoff and Dental Assistant
4. Plaque removal - Hock and Lamont
5. Gingival index - Hock and Lamont
7. Runner - Dental assistant

June 23 appointments at 3:30, 4:00, 4:30, 5:00, 5:30
June 30 appointments at 1:00, 1:30, 2:00, 2:30, 3:00

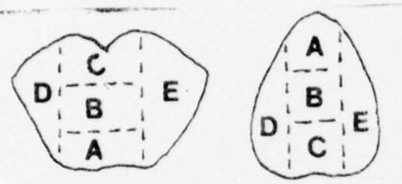
Appendix F - Example of Data Collection Form - <u>In</u> <u>Vivo</u> Study

No. 8

Name Fred Insogna

Date 23

Plaque Index



Tooth		A	B	C	D	E	Total
3	F	✓			✓	✓	3
	L				✓	✓	2
9	F				✓		1
	L				✓		1
12	F				✓	✓	2
	L				✓	✓	2
19	F				✓		1
	l				✓		1
25	F						—
	L				✓	✓	2
28	F				✓		1
	L	✓			✓	✓	3
TOTAL							19

Tooth		Gingival Index
3	B L	1 1 d m 1 1
9	B L	0 0 m d 0 0
12	B L	0 0 m d 1 0
19	B L	1 1 m d 1 1
25	B L	0 1 d m 1 0
28	B L	0 0 d m 1 0
Mean Score		12

0.50

1. Subjective Staining NO
2. Gindex score 85 75
3. Plaque weight 4.0
4. Total microorganisms 13, 5.3
5. Comments "gingiva seem whiter"

Frequency Scores for GI
- 0 - 12
- 1 - 12
- 2 - 0
- 3 - 0

Appendix G - Participant's Subjective Symptoms

2.	D.F.	23 - #1 yes; #5 "gums turned white" teeth - yellow 30 - #1 previous wk, yes; #5 plaque on buccal gingiva - difficult to see gingival color.
3.	R.W.	23 - #5 "gingiva swollen" according to patient. Tooth 9: crown
4.	D.H.	23 - #5 "teeth looked yellower"
7.	P.V.	30 - a lot of bleeding during plaque removal
8.	F.I.	19 - Blood in sample. 23 - "gingiva seem whiter"
11.	S.H.	23 - *Hellden, feel granular. 30 - "teeth look dark" tongue stained
12.	R.A.	23 - "gingiva whiter"
13.	K.R.	23 - *Hellden
14.	D.W.	23 - stained tongue. Tooth 19: 2nd molar
15.	J.G.	23 - #5 "feels gritty" Tooth 9: crown
16.	R.L.	23 - PI done prior to G.I., so G.I. done at stain present
17.	R.M.	30 - stained tongue and teeth. Tooth 12: rotated (buccal is distal)
18.	G.R.	23 - Tooth 12: 2nd premolar
19.	R.D.	23 - *Hellden; "gingiva looked whiter, swollen , warm."
20.	R.V.	19 - Still traces of disclosing solution at 3:30 (used at 12:00)/23 - "hot spots felt on gingiva"
21.	T.C.	30 - stained tongue and teeth
23.	D.D.	23 - Tooth 12: recession on buccal
25.	G.B.	23 - #1 Dark yellow (?) - plaque 30 - erupting 3rd molar, lower right
26.	B.R.	23 - #5 - staining of tongue. 30 - #5 - brown tongue
27.	A.H.	23 - *Hellden. 30 - stained tongue and teeth
28.	S.I.	23 - *Hellden 30* Hellden collected plaque
29.	M.R.	30 - # 1 stained teeth. Tooth 12:recession on buccal
30.	G.N.	23 - Tooth 3: none; substitute 4
31.	J.N.	30 - *Hellden plaque count; stained teeth and tongue. Tooth 12: 2nd premolar

Appendix H

DISTRIBUTION LIST

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